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THE EFFECT OF AVOCADO (*PERSEA AMERICANA* MILL.) LEAVES EXTRACT TOWARDS THE MOUSE'S BLOOD GLUCOSE DECREASE WITH THE GLUCOSE TOLERANCE METHOD

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ABSTRACT

Decrease in blood glucose level test with the use of oral glucose tolerance method from Avocado leaves was carried out. Swiss Webster male mice were divided randomly into five groups. They were negative control, positive control (Glipizide 0.013 mg/20 g BW) and three test groups treated with dosage of avocado leaves ethanol extract 0.490 g/kg, 0.980 g/kg and 1.960 g/BW respectively. Experiment was begun with feeding the mice with the test solution followed by feeding glucose solution (1.5 g/kg BW) 30 minutes later. Blood glucose levels were assessed using glucometer kit, from zero to 3 hours, at ½ hourly interval. These results analysed by one way ANOVA showed there were significant difference ($p < 0.05$) between Ethanol extract of avocado leaves treated with mice (0.490, 0.980 g/kg BW) and control groups. Furthermore, when Tukey's test was performed, avocado treated mice (1.960g/kg BW) reduce glucose level to 64.27%. The effectiveness of this treatment was not significantly different to those treated with glipizide (68.50%).

INTRODUCTION: In Indonesia, the laymen have used natural substances, especially from plant in order to prevent and cure diseases since a long time. The use of these natural substances is generally based on the empirical use, which is passed from generation to generation by the ancestors; however, the clinical data which guarantee the advantages and safety of traditional medicine are still very limited. The use of traditional medicine needs to be developed with a pre-clinical test and a medicine safety test so that the quality of traditional medicine is guaranteed. One of the advantages of traditional medicine is, among others, to cure diabetes mellitus.

Diabetes Mellitus is a disease due to metabolic disorders marked by the high sugar level in the blood because of insulin deficiency or insulin effectivity decrease.

The lack of insulin secretion causes the blood glucose level to increase and exceed the normal limit of the glucose amount which is supposed to be in the blood. The metabolic disorders in diabetes mellitus will influence the body metabolism, such as the metabolism of carbohydrate, protein, and fat which can cause cellular damage in the body tissues^{1,2}.

When not managed well, diabetes mellitus will cause complications to other organs which can be fatal, therefore, it is really need to pay attention to and improve diabetes mellitus handling and treatment. One of the traditional medicine which can be used as an antidiabetic herb is *Persea americana* Mill., known as avocado or in Indonesia we called Alpukat. Based on some information, empirically avocado can decrease the blood glucose level.

This has been proven through the previous research which shows the activity of avocado seed ethanol extract as an antidiabetic agent ³. Based on the consideration above, the test to discover the effects of giving avocado towards the decrease of glucose level in the blood is conducted. In this research, avocado leaves are used as antidiabetic herbs. The presence of abnormality in the glucose metabolism can be discovered with oral glucose tolerance.

The research is conducted to discover the potential of the avocado leaf ethanol extract to reduce the blood glucose level of mice by using the oral glucose tolerance method. From this research, it is expected that the results can increase the effort of society's health towards the traditional medicine development, and it is also expected that the results can provide scientific information for common people concerning avocado leaves as alternative medicine for curing diabetes mellitus. Extraction towards avocado leaf powder is done by using an ethanol solvent, where the thick extract of avocado leaf ethanol obtained is done with phytochemical screening and the potential test in decreasing the blood glucose level.

The avocado leaves ethanol extract test preparation is given with various dose levels orally to a group of mice. The hyperglycemia effects from the test preparation are seen by measuring the blood glucose level of the mice with the interval of 0.5 hour starting from the 0 hour to the third hour by using a glucometer (measurement for the blood glucose level) ⁴.

MATERIAL AND METHOD

Material: Avocado leaves were taken from Balitro, Cimanggu, Bogor., male mice with Webster Swiss sp. Glucose, glipizide, agua destilata, picric acids, CMC Na 0,5%.

Method:

Ethanol extract of Avocado leaf: The avocado leaves powder is obtained by drying the fresh avocado leaves in the open air to let the leaves be blown by the wind and become dry, and then they are put into a blender until the avocado leaves dry powder is obtained as much as 500 g; then it is macerated with 70% of ethanol for 24 hours, and extraction is done 3 times before being filtered. The filtrate obtained is

concentrated with a rotavapor so that thick extract is obtained and then kept in a clean container which is sealed tight ⁵.

Hypoglycaemic mice: The hypoglycaemic mice were done by giving orally with 1.5 g/kg BW glucose ⁶.

Measurement of Blood Glucose Level: The blood glucose level was measured using glucometer in the fasting mice. Before cutting the tail, wrap the tail use alcohol for dilatation.

Determining the Dose: The glipizide dose for adults is: 5 mg/70 kg BW ⁷.

Conversion factor: 0.0026⁸ so the glipizide dose for a mouse is: 5 x 0.0026 = 0.013 mg/20 g of the mouse's weight.

The dose for the avocado leaf ethanol extract test preparation: The testing of the blood glucose level decrease from the avocado leaf ethanol extract is conducted with the oral glucose tolerance method. To discover which dose would have the potential to decrease the blood glucose of the mice, the experiment applied three different doses as follows :

- The group given the avocado leaf ethanol extract with the dose of 0.490 g/kg BW;
- The group given the avocado leaf ethanol extract with the dose of 0.980 g/kg BW;
- The group given the avocado leaf ethanol extract with the dose of 1.960 g/kg BW.

The working procedure ⁴: There were 30 mice which were adapted first for one week. All mice were made to fast for more or less 20 hours. Afterwards, the mice were weighed to determine the glucose dose and then the avocado leaf ethanol extract was divided into 5 groups in random; each group consisted of six mice:

- the negative control group (CMC Na 0.5%);
- the positive control group (glipizide);
- the group given the avocado leaf ethanol extract with the dose of 0.490 g/kg BW;
- the group given the avocado leaf ethanol extract with the dose of 0.980 g/kg BW;
- the group given the avocado leaf ethanol extract with the dose of 1.960 g/kg BW.

Next, in the early phase the blood of the mice was taken through the lateral vena before given the test preparation, and at the beginning the blood glucose level measurement used a glucometer. Afterwards, each group was given the test preparation orally in accordance with its group.

After a half an hour post the treatment, they were given glucose solvent with the dose of 1.5 g/kg BW. Then the blood glucose level of the mice in each group was measured by using a glucometer with the interval of 0.5 hour starting from the 0 hour to the third hour after they were given glucose.

RESULTS:

Rendemen of the Extract: From this study the rendemen results of extraction was 20.15%

Phytochemical Screening: The phytochemical screening via Farnsworth method⁹ was conducted using crude powder and the ethanol extract. In crude powder of Avocado leaves presence Flavonoid, saponin, steroid/triterpenoid and coumarin. The results of . ethanol extract presence Flavonoid, saponin and coumarin. The results of phytochemical test was shown in **Table 1**.

Blood Glucose Level: Based on the analysis with the one-way ANOVA statistical method continued with the Tukey test, at the first hour, the 1.5 hour, the second hour, and the 2.5 hour, there was no significant difference among the groups. At the third hour there was a significant difference among the negative control group, the group given the dose of 0.490 g/kg BW, and the group given the dose of 0.980 g/kg BW from the positive control group (glipizide) and the group given the dose of 1.960 g/kg BW.

The decrease percentage for each group, the negative control group, the positive control group, the group given the dose of 0.490 g/kg BW, and the group given the dose of 0.980 g/kg BW, and the group given the dose of 1.960 g/kg BW, is 60.07%, 68.50%, 61.38%, 53.69%, and 64.27% respectively. Based on the above percentage, the group given the dose of 1.960 g/kg BW had more potential in decreasing the blood glucose

level if compared with the group given the dose of 0.490 g/kg BW and the group given the dose of 0.980 g/kg BW. The results was shown in **figure 1**.

DISCUSSION: Our study results showed that the phytochemical screening of Avocado leaves crude power and ethanol extract were flavonoid.

TABLE 1: PHYTOCHEMICAL SCREENING OF AVOCADO.LEAVES

Chemical content	Powder	Ethanol extract avocado leaves
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tanin	-	-
Quinon	-	-
Steroid/Triterpenoid	+	-
Volatile oil	-	-
Coumarin	+	+

+ : positive reaction; - : negative reaction

Flavonoids are found a lot in a plant and have several functions, among others, as a producer of yellow, red, and blue pigments on flowers. Flavonoids can be extracted with 70% of ethanol and will remain in the water layer after this extract is mixed with hydrocarbon.

Flavonoids also has effects of antitumor, immunostimulants, analgesics, anti-inflammatory, anti-virus, antibacterial, antidiarrhoeal, antihepatotoxic, antihyperglycemic, and as a vasodilator.

Saponins are the soap-like foaming solutions and classified by the aglycone structure in the steroid/triterpenoid. Those two chemical compounds have the effects of anti-inflammatory, analgesics, and cytotoxicity. Cumarin is aetheric chemical compound causing the emergence of unique fragrance having an effect as insecticide^{10, 11}.

The chemical compound influencing the blood glucose level decrease from the avocado leaf ethanol extract is assumed to be flavonoids¹¹.

The Comparison of the Blood Glucose Level: The measurement of the blood glucose level using glucometer was showed in the **figure 1**.

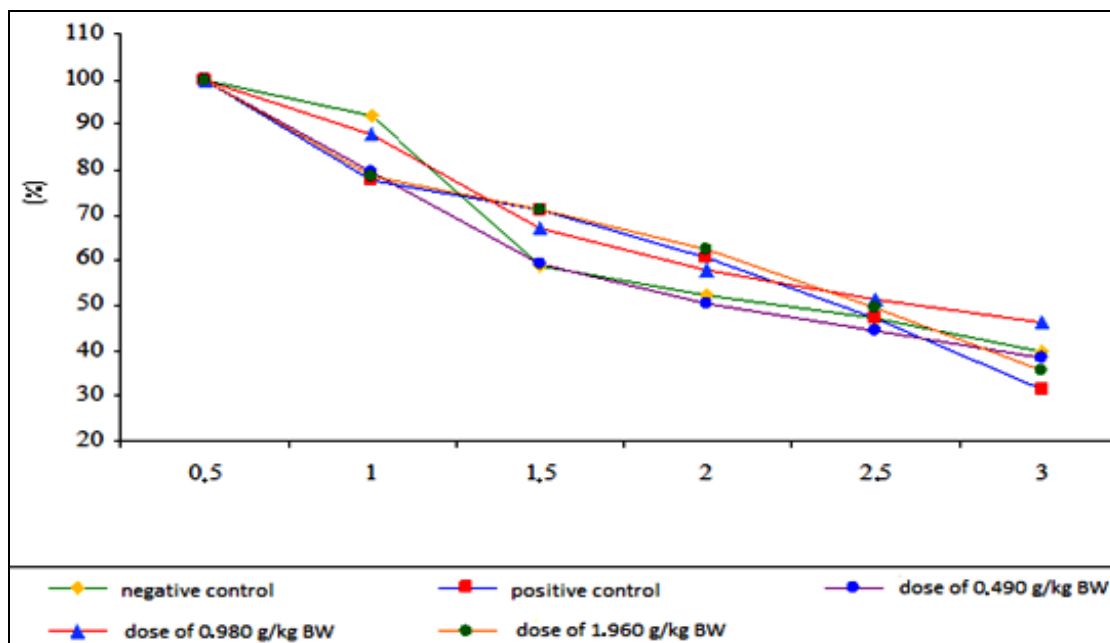


FIGURE 1: THE COMPARISON OF THE BLOOD GLUCOSE LEVEL

The Tukey Test shows that the avocado leaf ethanol extract has an effect to decrease the blood glucose level. The effect often occurs along with the increase of the dose. If compared with the group given avocado leaves with the dose of 1.960 g/kg BW, it seems that the hypoglycemia effect of this preparation group occurred the strongest and there was no significant difference from the positive control group; it happens probably because the test preparation worked by stimulating the insulin secretion so that it could increase the glucose transport through cell membranes. The glucose transportation speed through cell membranes is very much influenced by the amount of insulin secretion. When insulin is excreted in a large amount by the pancreas β cell, the glucose transport speed through the cell membranes can achieve 10 times faster than the speed when there is no insulin secretion¹².

In the previous research, the part of the avocado which was used to reduce the blood glucose level is the avocado seed. Meanwhile, in this research what would be tested is the effect of giving avocado leaves towards the blood glucose level decrease, as avocado leaves are more easily obtained. To discover which dose would give the potential to reduce the blood glucose level from the avocado leaf ethanol extract, the choices of dose in this research were based on the dose which can reduce the blood glucose level from the avocado seed ethanol extract, which is the dose of 0.980 g/kg BW(3). The dose choices for the avocado leaf ethanol

extract apply three different doses, which are the dose which is reduced twice lower than the dose having the potential from the avocado seed ethanol extract, the dose having the potential from the avocado seed ethanol extract, and the dose which is increased twice higher than the dose having the potential from the avocado seed ethanol extract.

Thus, there were the group given the avocado leaf ethanol extract with the dose of 0.490 g/kg BW, the group given the avocado leaf ethanol extract with the dose of 0.980 g/kg BW, and the group given the avocado leaf ethanol extract with the dose of 1.960 g/kg BW. It can be seen whether or not the dose having the potential from the avocado leaf ethanol extract is similar with the avocado seed ethanol extract in decreasing the blood glucose level.

From this research, the avocado leaf ethanol extract with the dose of 1.960 g/kg BW has a more potential activity in decreasing the blood glucose level if compared with the avocado leaf ethanol extract with the dose of 0.490 g/kg BW and the avocado leaf ethanol extract with the dose of 0.980 g/kg BW. It is discovered that the dose having the potential in decreasing the blood glucose level from the avocado leaf ethanol extract is the dose which is increased twice higher than the dose having the potential in decreasing the blood glucose level from the avocado seed ethanol extract.

CONCLUSION:

1. The rendement of the extract of Avocado leaf was 20.15 %
2. The Phytochemistry test of the powder Avocado were Flavonoid, asaponin, steroid/triterpenoid and coumarin.
3. The extract of Avocado leaf with the dose 1.960 kg BW can reduce the blood level of the mice 64.27 %. There was no significant difference level compare to the positive control glipizid 68.50%.

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